CHEMICAL MIMICRY AS AN INTEGRATING MECHANISM FOR THREE TERMITOPHILES ASSOCIATED WITH *RETICULITERMES VIRGINICUS* (BANKS)[1,2]

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Introduction

The mechanisms by which termitophiles integrate themselves into the social life of termite colonies have long intrigued entomologists (Kistner, 1979). Various authors have suggested that plausible integration mechanisms might include the using of "appeasement chemicals" (Wilson, 1971), passing as morphological mimics (Kistner, 1968), or engaging in behavioral mimicry (Kistner, 1979). We recently reported (Howard et al., 1980a) that the host-specific, highly integrated termitophile *Trichopsenius frosti* Seevers associated with *Reticulitermes flavipes* (Kollar) possesses the same complex mixture of cuticular hydrocarbons as its termite host. We suggested that this was an example of chemical mimicry which functioned to integrate this beetle into the termite society.

Reticulitermes virginicus (Banks) is sympatric with *R. flavipes* throughout much of its range and, as predicted (Howard et al., 1978; Blomquist et al., 1979), the two species possess distinctly different cuticular hydrocarbons which function as species recognition cues (Howard et al., 1982). They also have different termitophilous cohorts. Thus, *T. frosti* is associated only with *R. flavipes* whereas *T. depressus* Le Conte, *Xenistusa hexagonalis* Seevers (both Staphylinidae: Trichopseniinae), and *Philotermes howardi* Kistner and Gut (Staphylinidae: Aleocharinae) are associated only with *R. virginicus*. We now report that the three *R. virginicus* staphylinids also appear to use chemical mimicry as an integrating mechanism; i.e.,

[1]*Manuscript received by the editor June 3, 1982.*

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they have the same complex mixture of cuticular hydrocarbons as their host termite. In addition, we report that at least one of these beetles *(X. hexagonalis)* biosynthesizes its hydrocarbons.

Methods and Materials

Portions of several colonies of *R. virginicus* were collected in 1979 from pine logs in Harrison, Jackson, and Stone Counties, Mississippi. The beetles were separated from the termites, counted by species, and stored at −20° C until used. A total of 230 beetles was collected: 10 *P. howardi,* 140 *T. depressus,* and 80 *X. hexagonalis.* Cuticular hydrocarbons from pooled samples (by species) were isolated and separated as previously described (Howard et al., 1978). Hydrocarbons were characterized by gas-liquid chromatography (GC) retention times and by electron impact (EI) and chemical ionization (CI) mass spectrometry (Howard et al., 1980b; Jackson and Blomquist, 1976). Double bond stereochemistries were determined by comparison with standards using argentation thin-layer chromatography ($AgNO_3$–TLC) (Kates, 1972).

In vitro biosynthesis experiments were conducted as previously described (*Howard et al.,* 1980a) using 60 *X. hexagonalis* collected from a single colony of *R. virginicus* in September 1979.

Radioactivity was assayed by liquid scintillation counting for 10 minutes at about 85 percent counting efficiency. All counting was done with a standard deviation of less than 5 percent. A portion of the isolated hydrocarbons was assayed for total radioactivity. The remainder of the material was separated by $AgNO_3$–TLC into saturated, monounsaturated, and diunsaturated components, which then were assayed for radioactivity.

Results

The retention times of all peaks present in the GC profile of cuticular hydrocarbons from *R. virginicus* (Fig. 1) match those from the GC profile of the cuticular hydrocarbons of *P. howardi* (Fig. 2), *T. depressus* (Fig. 3), and *X. hexagonalis* (Fig. 4). Confirmation of the chemical identity for each of the hydrocarbon components in most of the GC peaks was obtained by EI and CI mass spectrometry (MS). In every instance, the GC-MS retention times and mass spectra of the beetle hydrocarbon components were identical to those

previously obtained from *R. virginicus* cuticular hydrocarbons (Howard et al., 1982). Likewise, concurrently obtained $AgNO_3$–TLC retention values (Rf) were identical for all beetle derived alkenes and *R. virginicus* alkenes. Components which were identified include n-alkanes, 2-, 3-, 11-, 13-, and 15-methylalkanes, 11,15-dimethylalkanes, Z-9-alkenes, Z,Z-7,9-dienes, and E/Z-6,9-dienes ranging in carbon number from C_{21} to C_{40} (Table 1). Double bond location and stereochemistries of the beetle derived alkenes were inferred solely from GC and GC-MS retention time data, and $AgNO_3$–TLC Rf data, since insufficient sample was available for infrared analysis and methoxymercuration-demercuration (Blomquist et al., 1980). Early eluting components not identified by a number in Figures 1 to 4 are unidentified, but have retention times consistent with a homologous series of *n*-alkanes.

The relative abundance of individual hydrocarbon components varied from species-to-species, but no more so than that of their termite host, whose percent composition varies considerably by caste (Howard et al., 1982).

The *in vitro* radioisotope incorporation experiment was conducted with *X. hexagonalis* to determine if this species can biosynthesize its cuticular hydrocarbons *de novo*. Howard (1978) reported that this species engages in frequent allogrooming with its termite host, with the resulting possibility of acquiring host hydrocarbons by mechanical transfer rather than by *de novo* biosynthesis. A combination of these two alternatives is also possible. After 2 hours of incubating beetle cuticular tissues with 10 μCi of [1−^{14}C]-acetate, 19.6 ± 8.8 pmole (mean ± SD) of [1−^{14}C]-acetate was incorporated into hydrocarbon. About 87.8 ± 5.3 percent of the radioactivity was in the alkane fraction, 10.2 ± 4.0 percent was in the alkene fraction, and 1.9 ± 1.3 percent was in the alkadiene fraction. This closely approximates the distribution of alkanes and olefins in *X. hexagonalis*, suggesting that this species can *de novo* biosynthesize its cuticular hydrocarbons. *In vitro* biosynthesis experiments were not conducted with *T. depressus* and *P. howardi* because we were unable to collect enough beetles simultaneously.

Discussion

The striking mimicry of hydrocarbon components observed among these three beetles (representing two subfamilies) and their

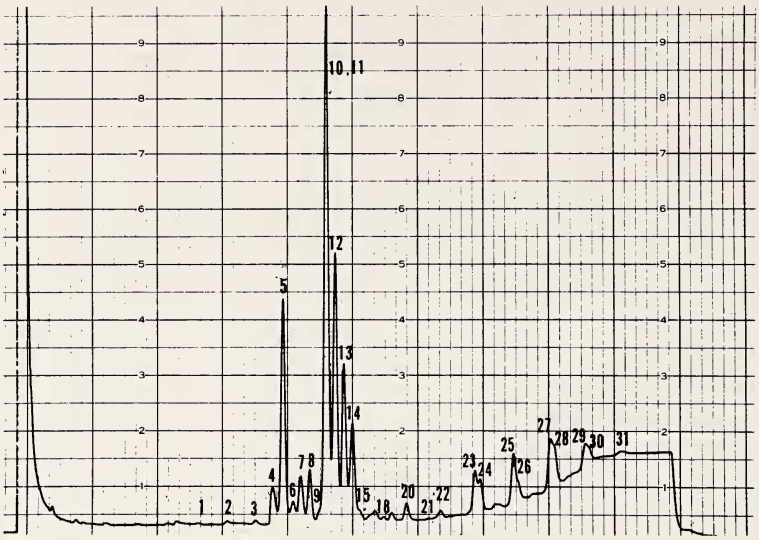

Fig. 1. GC trace of total cuticular hydrocarbons of *Reticulitermes virginicus*. GC conditions: 1.83 m × 3 mm i.d. Stainless steel column packed with 3 percent (w/w) SP-2100 on 100/120 mesh Supelcoport; temperature programmed from 150° to 325°C at 5°C/min.

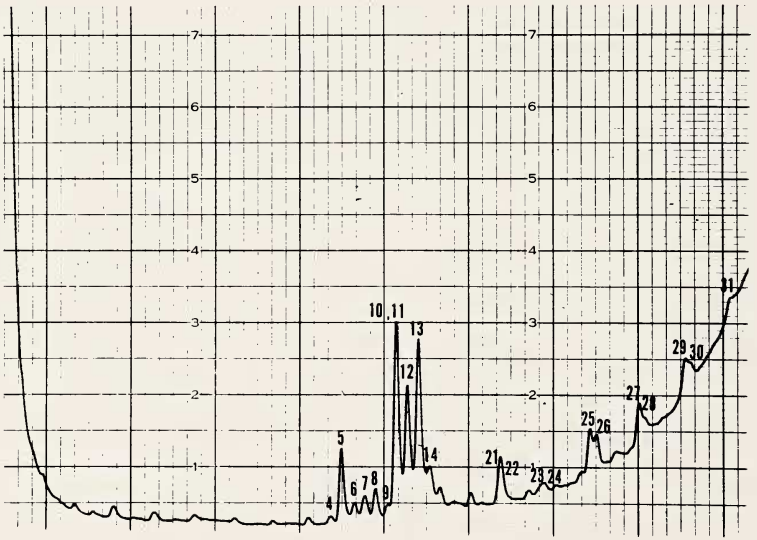

Fig. 2. GC trace of total cuticular hydrocarbons of *Philotermes howardi*. GC conditions same as for Fig. 1.

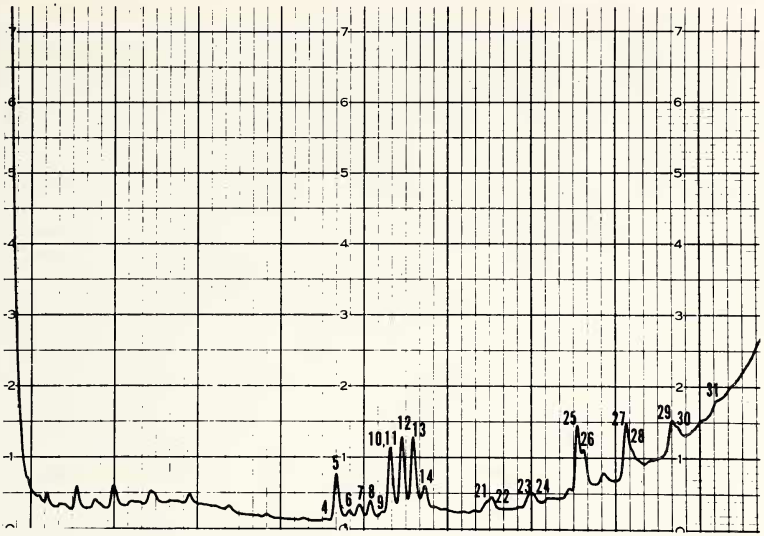

Fig. 3. GC trace of total cuticular hydrocarbons of *Trichopsenius depressus*. GC conditions same as for Fig. 1.

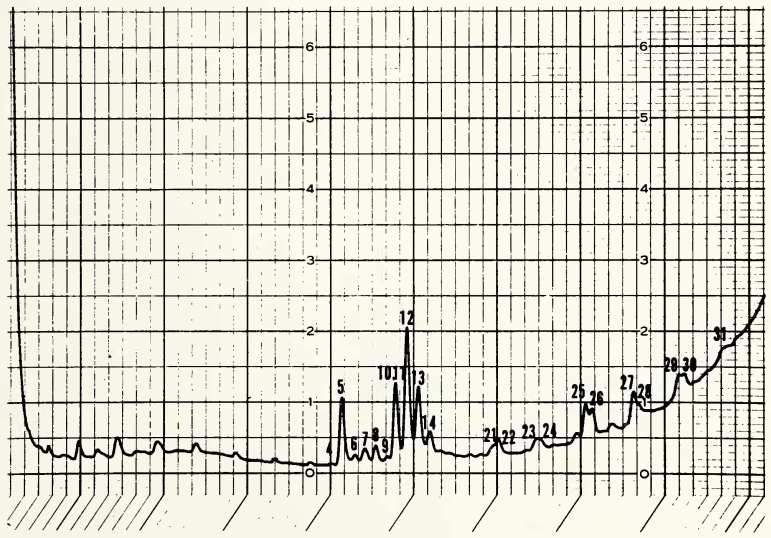

Fig. 4 GC trace of total cuticular hydrocarbons of *Xenistusa hexagonalis*. GC conditions same as for Fig. 1.

termite host is strongly suggestive for their role as integrating factors. It also supports our earlier hypothesis that cuticular hydrocarbons may serve as species recognition cues (Howard et al., 1978; Blomquist et al., 1979; Howard et al., 1980a; Howard et al., 1982). Behavioral evidence for this interpretation comes from the finding (Howard, unpublished observations) that live *T. depressus* placed into laboratory colonies of *R. flavipes* were killed by the termites within a 24-hour period (five observations). Similarly, the placing of live *T. frosti* into laboratory colonies of *R. virginicus* results in their being killed (five observations). Beetles can be freely exchanged among different colonies of their hosts however. These two *Trichopsenius* spp. are nearly identical morphologically and behaviorally, but differ markedly with respect to cuticular hydrocarbons. Similar transplants of workers or soldiers of *R. flavipes* or *R. virginicus* into colonies of the other species also resulted in the death of the alien individual (five observations). Transplants of conspecific termites into different colonies did not produce agonistic interactions (five observations). As with the beetles, the two termite species are morphologically and behaviorally quite similar. We have shown that *R. virginicus* workers are antagonistic towards neutral, critical-point dried (CPD) conspecific workers treated with *R. flavipes* cuticular hydrocarbons (Howard et al., 1982), but are not aggressive toward CPD workers treated with *R. virginicus* cuticular hydrocarbons. While we cannot exclude the possibility of other biochemical differences among either the beetles or their host termites, GC comparisons of total body extracts revealed none.

The termitophiles associated with *R. virginicus* (in common with other termitophiles) possess many epidermal glands (Kistner, 1979) which have often been postulated to be a source of chemicals which in some manner aids in the integration of the beetles into the termite society. While we cannot rule out this interpretation, we would like to suggest an alternative hypothesis for the function of these glandular products. Termitophiles are never found in great abundance (Wilson, 1971; Kistner, 1979), and the nature of termite nest-galley systems is such as to present substantial problems in the location and recognition of conspecifics. Perhaps these glands are producing pheromones directed at conspecifics rather than kairomones directed at their host. Since pheromones are usually produced in extremely

small amounts, such an interpretation would explain the lack of GC evidence to date for beetle derived biochemicals different from those of their termite host. An experimental test of this hypothesis must await the development of suitable bioassays.

Reticulitermes virginicus and its termitophiles have been co-evolving for a long period of time (Kistner, 1968, 1979). The beetles are totally integrated into the social life of the colony and appear to be chemically indistinguishable from the termites (chemical mimicry) vis-á-vis their cuticular hydrocarbons. Most known termite-termitophile associations, however, occur within the family Termitidae (Kistner, 1979). These associations are characterized by termitophiles ranging in status from nonintegrated to totally integrated. If our hypothesis is correct regarding the integrating role of cuticular hydrocarbons then a corresponding spectrum of congruences of hydrocarbon profiles would be predicted among the termitophiles of these communities. We are presently testing this hypothesis.

Many species of ants are known to have inquilines associated with them, but unlike termitophiles, these myrmecophiles are seldom host specific (Wilson, 1971). In addition, myrmecophiles seem to show a wider range of integration (or lack thereof) than do termitophiles. A correspondingly greater range of integrating mechanisms might therefore be expected, and have been found. These include body color, appeasement substances, trichomes, unicellular epidermal glands, physogastry, exudatoria and grandular antennae. All have been superbly reviewed by Wilson (1971) and Kistner (1979). The most recent addition to this plethora of mechanisms is the finding that the scarab beetle *Myrmecaphodius excavaticollis* (Blanchard) associated with various *Solenopsis* spp. ("fire ants") has a cuticular hydrocarbon composition which closely mimics that of its current ant host (Van der Meer, personal communication in Howard and Blomquist, 1982). The mechanism by which the beetles achieve this is unknown. Each of the four ant hosts that the scarab beetles is found with, however, has a unique hydrocarbon profile. Perhaps ants, like subterranean termites, also use cuticular hydrocarbons as species-recognition cues. Clearly a great deal remains to be learned before we achieve an adequate understanding of the diversity of relationships between social insects and their guests.

Table 1. Cuticular hydrocarbons of *Reticulitermes virginicus, Philotermes howardi, Trichopsenius depressus* and *Xenitusa hexagonalis.*

GC Peak[1]	Component	Carbon number[2]	Diagnostic MS ions[3]
1	n-C_{21}	21	296
2	n-C_{22}	22	310
3	11-MeC_{22}	23	168/169, 182/183, 324
4	Z-9-C_{23}	23:1	322
4	E/Z-6,9-C_{23}[4]	23:2	320
5	n-C_{23}	23	324
6	11-MeC_{23}	24	168/169, 196/197, 338
7	2-MeC_{23} + 3-MeC_{23}	24	294/295, 322/323, 338; 280/281, 308/309, 338
8	n-C_{24}	24	338
9	11-Me + 13-MeC_{24}	25	168/169, 210/211, 352; 182/183, 196/197, 352
10	2-MeC_{24}	25	308/309, 336/337, 352
11	E/Z-6,9-C_{25}	25:2	348
11	Z-9-c_{25}	25:1	350
12	n-C_{25}	25	352
13	11-Me + 13-MeC_{25}	26	168/169, 224/225, 366; 182/183, 210/211, 366
13	Z,Z-7,9-C_{25}	25:2	348
14	2-Me + 3-MeC_{25}	26	322/323, 350/351, 366; 308/309, 336/337, 366
15	n-C_{26}	26	366
16	11-Me + 13-MeC_{26}	27	168/169, 238/239, 380; 182/183, 210/211, 380

Table 1. Continued

GC Peak[1]	Component	Carbon number[2]	Diagnostic MS ions[3]
17	2-Me + 3-MeC$_{26}$	27	336/337, 364/365, 380; 322/323, 350/351, 380
18	n-C$_{27}$	27	380
19	11-Me + 13-MeC$_{27}$	28	168/169, 252/253, 394; 182/183, 224/225, 394
20	n-C$_{28}$	28	394
21	11-MeC$_{28}$	29	168/169, 266/267, 408
22	n-C$_{29}$	29	408
23	11-MeC$_{29}$	30	168/169, 280/281, 408
	11-Me + 13-Me + 15-MeC$_{31}$	32	168/169, 308/309, 450; 182/183, 280/281, 450; 224/225, 252/253, 450
24	11,15-diMeC$_{31}$	33	168/169, 238/239, 252/253, 322/323
25	11-Me + 13-MeC$_{33}$	34	168/169, 322/323; 182/183, 294/295
26	11,15-diMeC$_{33}$	35	168/169, 238/239, 280/281, 350/351
27	11-Me + 13-Me + 15-MeC$_{35}$	36	168/169, 350/351; 182/183, 308/309; 224/225, 280/281
28	11,15-diMeC$_{35}$	37	168/169, 238/239, 308/309, 378/379
29	11-MeC$_{37}$	38	168/169, 378/379
30	11,15-diMeC$_{37}$	39	168/169, 238/239, 336/337, 406/407
31	11-MeC$_{39}$	40	168/169, 406/407

[1]See Figures 1 to 4.

[2]Determined from CI-MS where $(M - 1)^+$ is always the base peak.

[3]EI-MS.

[4]E/Z-6,9-C$_{23}$ indicates E/Z-6,9-tricosadiene, where the slash indicates that one double bond is cis (Z) and one is trans (E), but which is which, is unknown. The diene in peak 11 is named correspondingly.

Summary

The three highly integrated staphylinid termitophiles (*Philotermes howardi* Kistner and Gut, *Trichopensius depressus* Le Conte, and *Xenistusa hexagonalis* Seevers) associated with *Reticulitermes virginicus* (Banks), possess the same cuticular hydrocarbons as their host. This congruence is hypothesized to be a form of chemical mimicry and is postulated to function as a major way these beetles achieve integration into the termite society.

Acknowledgement

G. J. Blomquist acknowledges the support of the Science and Education Administration of the U.S. Department of Agriculture under grant 7801064 from the Competitive Research Grant Office.

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